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THE EFFECTS OF HEAD TRAUMA AND BRAIN INJURY ON NEUROENDOCRINOLOGIC FUNCTION

ANNUAL REPORT

July 13, 1984

Paul D. Woolf, M.D.
Robert W. Hamill, M.D.
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Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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University of Rochester Medical Center
Rochester, New York 14642

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

During the first year of this contract, 79 patients have been studied, 37 of whom had acute severe brain injury following injury. In four patients, associated spinal cord injury or intracranial bleeds were present. Forty-two control patients having a variety of acute medical/surgical problems were followed. Attention has been focused on the use of catecholamines as predictors of patient outcome. We have determined that a gradient exists in sympathetic nervous system activation

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after head trauma indicating that peripheral neurohumoral markers, particularly norepinephrine reflect the extent of brain injury. It was also determined that markedly elevated norepinephrine levels within 48 hours of severe brain injury suggests little improvement in neurologic function within the first week of injury. In contrast patients with comparable neurologic deficits, but only modest elevated norepinephrine levels, have significant neurologic improvement.

Two different studies on the effects of brain injury on neuroendocrine function have been completed and a third started. In the first, it was found that patients with increased intracranial pressure and intact brain stem function failed to suppress circulating cortisol levels despite massive dosage of glucocorticoids. This is in contradistiction to patients with increased intracranial pressure and abnormal brain stem function and those in whom intracranial pressure was normal, all of whom had low cortisol concentrations. The patients with nonsuppressed cortisols had low and not high ACTH levels. Thus, our data suggest that: a) increased intracranial pressure is a stimulus for adrenocortical activation, b) we have identified for the first time a hormonal marker of brain stem dysfunction, namely that the brain stem modulate the adrenocorticol activation of increased intracranial pressure, and c) this appears to be a non-ACTH mediated event. The second study, in contrast, indicates that gonadal function is affected not only by traumatic brain injury, but by several other acute medical problems, suggesting that it is a generalized stress response. In this project, testosterone, LH, and FSH levels were followed in 54 subjects in the following categories: severe traumatic brain injury 17 men, 5 women; vascular brain injury 14 women; elective surgery 7 men; myocardial infarction 11 men. The median time between the acute event and the acquisition of the first sample was under six hours. In all patients who become critically ill, temporary hypogonadotropic gonadal insufficiency occurred, irrespective of etiology. This was manifested in the men by low testosterone and in women by low estrogen levels without compensatory increases in gonadotropins. Free testosterone levels also fell, but sex hormone binding capacity remained unchanged. Finally, in order to study the effects of brain injury on pituitary thyroid axis, a new method for the measurement of free T_4 has been developed and validated. This method is based on ultrafiltration technology and compares favorably with equilibrium dialysis methods which are generally available only in commercial laboratories. Studies are currently underway making use of this new assay.

The first year of the project can be divided into two interrelated parts--organizational and investigational. The former included familiarizing both the surgical and neurosurgical housestaff and attendings to the project, working out the logistics of sample aquisition and retrieval, improving our data information forms to make them more inclusive and easier to use for data entry and retrieval, developing assays for hormones that had not been performed on a routine basis and most importantly establishing a data base management system. The latter proved to be the most challanging. It was decided to use the VAX minicomputer with Ingress software for data entry and manipulation. SAS was chosen for data analysis. This combination offered the advantages of flexibility, ease of use and in house support without the need for an outside consultant.

STUDIES

A) Overview

From May 15, 1983 through May 14, 1984, 33 patients with head injury were followed. Four additional patients suffered head trauma with associated spinal cord injury (2) or intracranial bleed (2). Forty-two control patients were followed having the following medical problems: neurological--intracerebral bleed 7, subarachnoid hemorrhage 8, stroke 4, spinal cord injury 5, elective surgery 7 and acute myocardial infarction 11. Six patients in the head trauma group have enrolled in the longitudinal study of the effects of head trauma on long term pituitary function.

The following hormonal determinations were performed on these 79 patients: cortisol 952, ACTH 314, LH 437, FSH 397, testosterone 282, estradiol 79, 17 hydroxyprogesterone 36, androstenedione 36, dihydrotestosterone 36, DHEA sulfate 22, sex hormone binding globulin 144, growth hormone 40, TSH 40, prolactin 21, fractionated plasma catecholamines--norepinephrine, epinephrine, dopamine 414.

B Specific projects

As outlined in our proposal, we planned to 1) determine whether abnormalities of the sympathetic nervous system are a) related to specific brainstem, hypothalamic and cerebral lesions, b) responsible for increased patient mortality and morbidity and c) a reliable marker for brain death and 2) study the hypothalamic-pituitary axis both acutely to differentiate specific neurologic abnormalities from generalized "stress" responses and chronically to examine the role of acute brain injury on subsequent pituitary dysfunction.

1. Studies of sympathetic nervous system function

During the past year attention has been focussed on the use of catecholamines as predictors of patient outcome. Previous clinical studies of central nervous system injury indicated that heightened autonomic responses attend brain injury and may reflect the extent of brain injury and/or exert deleterious effects. The present investigations were designed to extend these studies and characterize the effects of brain injury on the activation of sympathoadrenomedullary and hypothalamo-pituitary-adrenal axes by utilizing plasma levels of norepinephrine, epinephrine, dopamine and cortisol. Comparison of the neurochemical indices with the extent of brain injury as reflected

by the Glasgow Coma Score (GCS) permitted clinical correlation. In general, severe traumatic brain injury (GCS 3-4, N=11) caused within 48 hours of injury a four- to five-fold elevation of norepinephrine (1306 ± 202 pg/ml [SE]) and epinephrine (240 ± 48 pg/ml), whereas plasma dopamine was only slightly increased (126 ± 37 pg/ml). Circulating cortisol levels were also increased (37 ± 3 µg/dl), reaching concentrations of >60 µg/dl in some patients. In contrast, in patients with relatively mild brain injury (GCS > 11), plasma catecholamines were only slightly elevated or remained within the normal range (norepinephrine: 484 ± 44 pg/ml; epinephrine: 91 ± 33 pg/ml, dopamine; 87 ± 18 pg.ml). Patients with marked (GCS 5-7) and moderate (GCS 8-10) brain injury exhibited indices between the two extremes, suggesting that **a gradient exists in sympathetic nervous system activation and that peripheral neurohumoral markers may reflect the extent of traumatic brain injury.** The correlation is best seen with norepinephrine: GCS 3-4, 1306 ± 202 pg/ml; GCS 5-7, 682 ± 104 pg/ml; GCS 8-10, 576 ± 114 pg/ml; GCS > 11, 458 ± 44 pg/ml.

In order to determine whether plasma norepinphrine values had prognostic value, initial norepinephrine levels in 11 patients with GCS 3-4 on entry were compared with their GCS at one week. In those patients whose GCS did not improve, the initial norepinephrine levels were markedly elevated (2176 ± 531 pg/ml), whereas patients who were GCS > 11 at one week had entry norepinephrine levels that were only slightly elevated (544 ± 89 pg/ml). **Therefore, markedly elevated norepinephrine levels reflected the extent of traumatic brain injury and had prognostic significance.** Conversely, a GCS of 3-4 was not necessarily associated with activation of the sympathoadrenomedullary or adrenocortical systems.

In order to determine whether these responses were specific for traumatic brain injury, 11 patients with nontraumatic brain injury including ischemic stroke and intracerebral and subarachnoid hemorrhage were studied. Although these patients also had elevated catecholamines, they exhibited a different pattern of response. Norepinephrine levels were elevated within 48 hours of admission, but in contrast to traumatic brain injured patients no correlation existed between the extent of brain injury and norepinephrine levels: GCS 3-4, 1073 ± 165 pg/ml; GCS 5-7, 1906 ± 418 pg/ml; GCS 8-10, 667 ± 88 pg/ml; GCS 11, 865 ± 192 pg/ml. In addition 3 patients with nontraumatic brain injury exhibited markedly elevated plasma dopamine levels (3630, 3648 and 711 pg/ml), while none of the patients with traumatic brain injury, regardless of GCS, had levels >366 pg/ml and the mean of 27 dopamine values from 11 patients with GCS 3-4 was only 92 ± 16 pg/ml. Although the mechanism(s) by which these different responses is unknown, our investigations of patients with traumatic or nontraumatic brain injury indicate that activation of the sympathoadrenomedullary and hypothalamo-pituitary-adrenal systems attend brain injury and that the profile of responses varies with the etiology.

Parts of these preliminary results will be reported at the 1984 meeting of the Society for Neuroscience and Fifth Annual Traumatic Head Injury Conference to be held at Braintree Hospital, Braintree, Mass.

2. Studies of the hypothalamic-pituitary axis

Two different acute studies on the effects of brain injury on pituitary function have been completed a) the effects of increased intracranial pressure on adrenocortical suppression by dexamethasone and b) the effects of brain injury on pituitary-gonadal function.

a) Adrenocortical secretion in acute brain injury

Under normal conditions adrenocortical secretion is regulated by ACTH and elevated endogenous or exogenous glucocorticoid levels depress the activity of this axis. However, it is now clear that these interactions are modified in several clinical conditions, such that the normal set point is altered causing a state of relative resistance to adrenal suppression. Pituitary dependent Cushing's disease and Nelson's syndrome are typical examples. In addition, patients with severe burns and with endogenous depression have elevated cortisol levels without apparent increases in ACTH.

In the investigation of the pituitary-adrenal axis in patients with severe traumatic brain injury resulting in coma, it became clear to us that cortisol concentrations were not universally suppressed despite treatment with dexamethasone in dosages of 16 to 64 mg/day. Because of these preliminary observations, a study was undertaken to determine whether there was a unique neurological abnormality or sets of abnormalities which could account for the loss of adrenocortical suppression. The results have been reported in the Journal of Clinical Endocrinology and Metabolism 57, 1245, 1983. Twenty-three patients in either the medical or surgical intensive care units of Strong Memorial Hospital were followed. Fourteen had acute head injury and nine had intracranial hemorrhage. All studies were initiated within 24 hours of admission. In 20 patients, the Glasgow coma score was 7 or less at the start of the study, i.e. vigorous stimulation failed to elicit eye opening, purposeful speech and purposeful movement. Neurologic function deteriorated to this level in 2 additional patients. For study purposes, patients were classified on the basis of intracranial pressure (ICP) and brain stem function. ICP was monitored in 16 patients and was considered to be elevated if monitored values were > 20 mm Hg or if computed tomography (CT) showed a mass lesion with a midline shift or compressed cerebral ventricles. Brain stem function was abnormal if pupillary abnormalities, decerebrate rigidity, abnormalities in the integration of extraoccular movements, including vestibular reflexes, or bilateral reflex abnormalities were present. Patients were then placed into one of four groups at the time of entry into the study: 1-normal ICP and brain stem function (n=7), 2-elevated ICP and normal brain stem function (n=4), 3-normal ICP and abnormal brain stem function (n=7), and 4-elevated ICP and abnormal brain stem function (N=5). In addition, 6 patients underwent a shift in their classification as a result of changes in their clinical status. The clinical characteristics of patients in each group at the time of study are presented in Table 1.

TABLE 1. Patient data at the time of study

	Group 1: normal ICP, normal BSF (n = 7)	Group 2: increased ICP, normal BSF (n = 6)	Group 3: normal ICP, abnormal BSF (n = 7)	Group 4: increased ICP, abnormal BSF (n = 9)
Mean age (yr)	45.9	38.3	32.9	30.8
Male/female	6/1	3/3	4/3	5/4
ICP monitored	3	2	5	6
Initial GCS ≤7	4	6	7	9
Trauma/brain hemorrhage	3/4	3/3	6/1	6/3
Outcome				
Recovered	6	2	0	2
Died	0	2	3	3
Vegetative	1	0	4	0

n includes patients whose classification changed. BSF, Brain stem function. ICP, intracranial pressure; GCS, Glascow coma score.

Dexamethasone was routinely given intravenously initially as a bolus and subsequently over 1 hr in doses of 4-16 mg every six hours from the time of admission. Plasma cortisol was measured daily or every other day from specimens drawn in the morning and at other times as frequently as possible. With our method morning cortisol levels in 19 normal subjects given 1 mg of dexamethasone the preceding midnight were below the level of detectability in 9 and averaged 1.2 ± 0.4 (± SD) µg/dl. Blood for ACTH was obtained in the morning in EDTA-containing tubes, chilled immediately, separated and frozen within 1 hr. The mean 0900 hours plasma ACTH level in 53 normal

subjects was 51.6 ± 27.7 (± SD) pg/ml, significantly greater than that in 19 normal subjects after 1 mg of dexamethasone at midnight (31.2 ± 16.8 pg/ml, $p < 0.005$).

Plasma cortisol levels declined to below 5 µg/dl in 3 noncomatose group 1 patients (GCS >7) within 12 hours of the first dexamethasone dose (Figure 1). However, 36 hours were required for suppression for the group as a whole. The t of the decline in cortisol was 18 hr, and the linear regression of circulating cortisol with duration of dexamethasone treatment was significantly correlated (Figure 1, $Y_{cortisol} = -1.1 X_{hours} + 40.0$, $r=0.55$ $p<0.05$). Because all subsequent cortisol levels remained below 6 µg/dl, comparative analysis of dexamethasone suppressibility among patient groups was performed only after the patient had been treated for at least 36 hours.

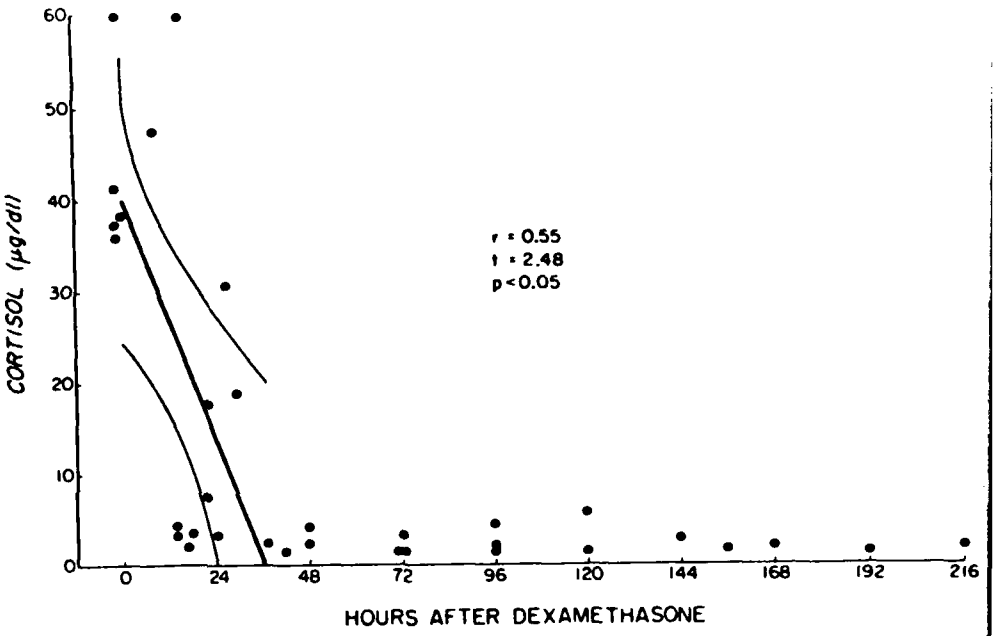

FIG. 1. Plasma cortisol levels during dexamethasone treatment (16–64 mg/day) in brain injured patients with normal ICP and brain stem function (group 1). The *heavy line* depicts the regression of plasma cortisol levels for the initial 36 h of dexamethasone therapy. The *curvilinear lines* are the 95% confidence intervals.

In contrast to the slow but normal cortisol suppression present in patients with normal ICP and brain stem function (group 1), patients with elevated ICP and normal brain stem function (group 2) had nearly universally elevated cortisol concentrations despite comparable dexamethasone treatment (Figure 2). Their mean cortisol value of

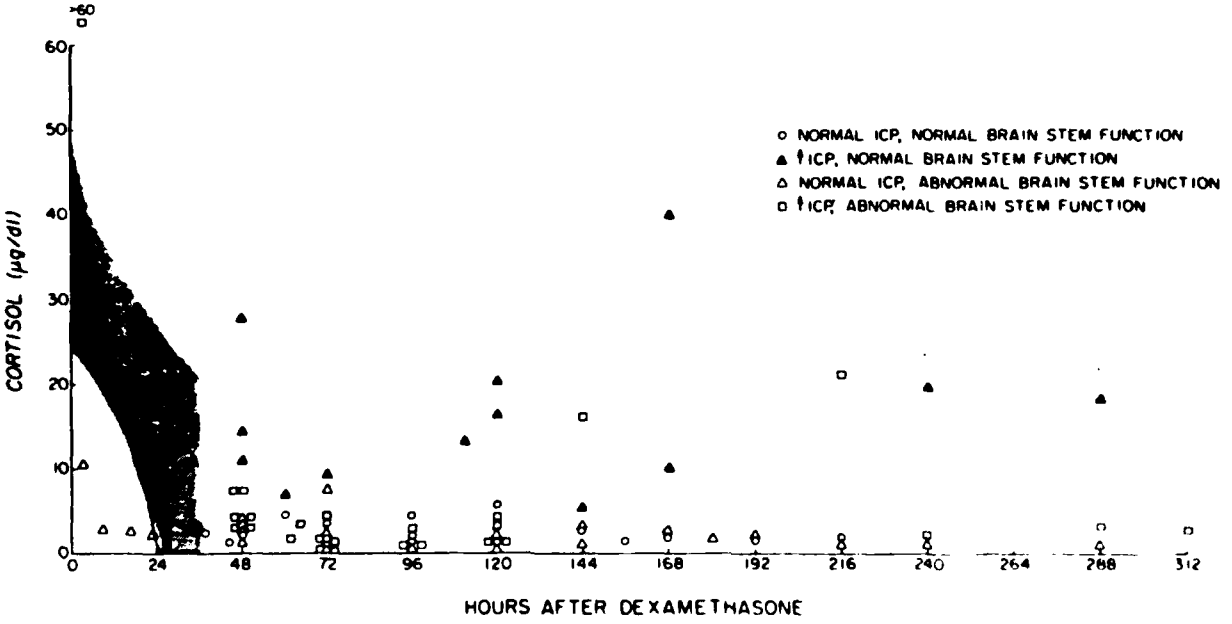

FIG. 2. Plasma cortisol levels in brain-injured patients during dexamethasone treatment. The *shaded area* represents the 95% confidence interv of the regression of plasma cortisol levels with time during dexamethasone therapy in group 1 (see Fig 1)

15.4 ± 2.6 (± SE) µg/dl was significantly greater than those in group 1 (2.4 ± 0.3 µg/dl $p<0.001$, Figure 3). The patients with normal ICP, but brain stem dysfunction (group 3) had complete cortisol suppression (2.1 ± 0.5 µg/dl, Figures 2 and 3) which was

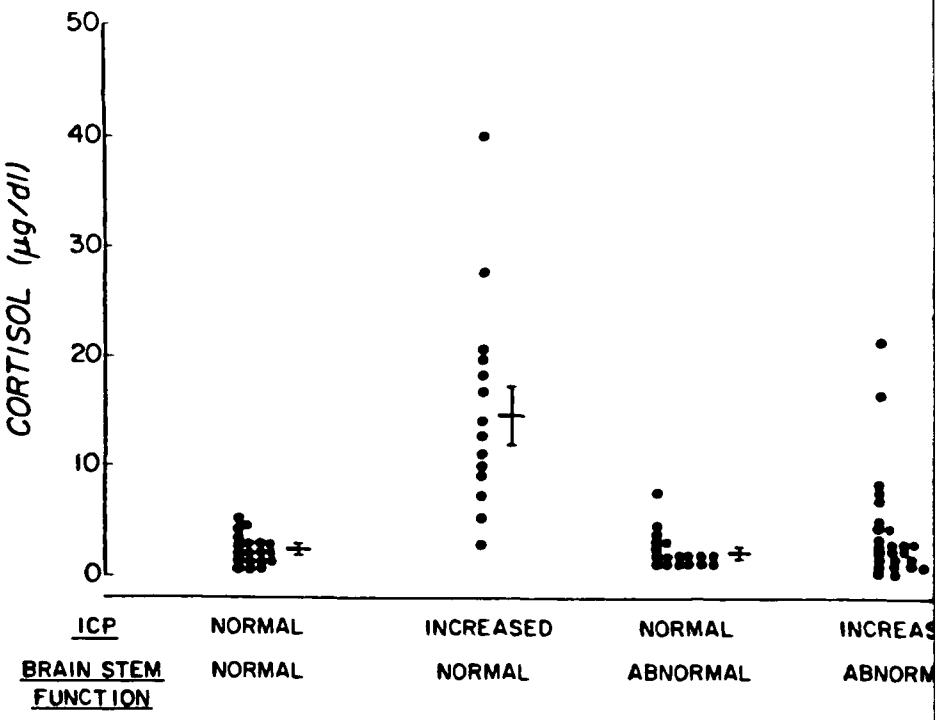

FIG. 3. Plasma cortisol levels after more than 36 h of dexamethasone treatment (16–64 mg/day). The cortisol data were placed in the appropriate clinical group based upon the patients' status at the time of blood withdrawal (see text). The numbers of samples per patient in each group were comparable.

comparable to group 1. Furthermore, when abnormal brain stem function was superimposed on elevated ICPs (group 4), cortisol levels were generally suppressed (3.9 ± 1.0 µg/dl) and significantly lower ($p < 0.001$) than in patients in whom brain stem function was normal (group 2, Figures 2 and 3). In the one patient with increased ICP whose brain stem function became normal, plasma cortisol levels were no longer suppressible. With the exception of 2 samples from one patient in group 4 who became septic, all cortisol values were below 8 µg/dl and generally less than 5 µg/dl.

Three patients were studied during and after the completion of temporal lobe herniation. During herniation they had elevated ICP and normal brain stem function and had no suppression of their cortisol levels. However, after herniation, cortisol suppression (levels <2 µg/dl) was present in the setting of brain stem dysfunction. Pituitary insufficiency was not responsible for the low cortisol levels since two patients had normal morning cortisol concentrations (16 and 12 µg/dl) after discontinuation of dexamethasone.

Simultaneous ACTH and cortisol levels were obtained in 31 samples from 14 patients without regard for the duration of dexamethasone treatment. The mean ACTH level was 45.6 ± 12.4 (± SE) pg/ml, not significantly different from that obtained between 0900 and 1000 hr in normal subjects (51.6 ± 3.8 pg/ml). There was no

correlation between ACTH and cortisol concentrations in any group (Figure 4) and only 3 samples from 1 patient were above the ACTH normal range. Despite the mean

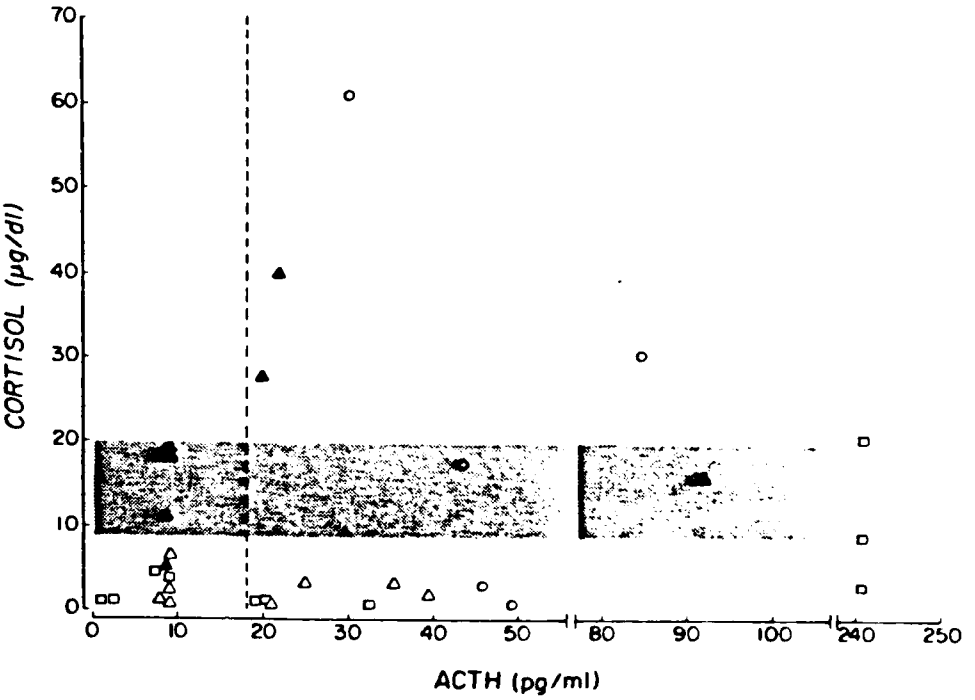

FIG. 4. Plasma ACTH and cortisol levels in all patient groups. ---, Lower limit of sensitivity for the ACTH assay. The *shaded area* represents the normal morning ranges for ACTH and cortisol (O, group 1; ▲, group 2; △, group 3; □, group 4; see text for definition).

nonsuppressed cortisol value of 15.4 ± 2.6 µg/dl in group 2, the mean ACTH level was 22.4 ± 10.0 pg/ml (n=8), which were significantly lower ($p < 0.01$) than those in normal subjects. Thus **we found a subset of patients with acute brain injury who failed to suppress pituitary-adrenocortical function despite massive dosages of glucocorticoids; i.e., those with elevated ICP and an intact brain stem function.** Patients with normal ICP have quantitatively normal adrenal suppression, although it appeared to occur more slowly than normal. However, because patients with abnormal brain stem function and elevated ICP had low, not high, circulating cortisol levels, it appears that the brain stem is involved in the mediation of the loss of dexamethasone suppression.

The mechanism for the profound adrenal stimulation in the patients with elevated ICP and normal brain stem function (group 2) is unclear. However, it was not due to excessive ACTH stimulation since the eight ACTH levels in the three patients studied from this group were not elevated and, in fact, were at or below the limits of assay sensitivity (18 pg/ml) in seven samples. Indeed, there were no significant differences between the ACTH concentrations in group 2 and those with suppressed cortisol levels (groups 1, 3 and 4) or between our entire study group and a normal population. Furthermore, the mean ACTH concentration of 22.4 ± 10.0 pg/ml in group 2 was similar to that at 0800 hours in normal subjects given dexamethasone (1 mg) the preceding midnight (31.2 ± 3.9 pg/ml, n=19). These data suggest, therefore, that adrenocortical activity is being stimulated either by an ACTH-independent mechanism or that adrenal responsivity to ACTH is increased. There is increasing evidence in the literature to support both possibilities.

Clearly the presence of a compromised brainstem negated the effect of elevated ICP on adrenal function. We cannot specify precisely which brain location is critical for mediation of adrenal stimulation under these circumstances. Abolition of intense

adrenal activation may be due to widespread diencephalic/mesencephalic/pontine damage or to a more localized lesion. **Nevertheless, for the first time a hormonal marker of brain stem dysfunction has been identified.** Obviously, the specific site(s) of brain stem effects on adrenocortical regulation are a matter of great interest and will require additional investigation of both the neurologic status and determination of a variety of neurologic evoked responses.

b. Pituitary-gonadal function and acute illness

The effects of systemic illness on the pituitary-thyroid axis have been extensively investigated. In contrast, alterations in gonadal function are less well recognized. There have been no systematic studies in men, and there are no data in women. Previous studies suggest that testicular function is altered by systemic illness, but the extent of these abnormalities and their pathogenesis have never been fully elucidated. Chronic brain injury, surgery, myocardial infarction, acute severe medical problems and respiratory insufficiency affect circulating testosterone levels. However, there is disagreement about whether this is an end organ problem or one that resides in the pituitary or higher centers, Studies of sex hormone binding capacity have also been conflicting. Consequently, we evaluated the effects of three critical illnesses on pituitary-gonadal function in both men and women: acute brain injury resulting in coma, myocardial infarction and elective surgery. The results of this study have been submitted for publication, and they will be presented at the Fifth Annual Traumatic Head Injury Conference to be held at Braintree Hospital, Braintree, Massachusetts.

The study population consisted of 54 subjects (35 men and 19 women). Their demographic data are shown in Table 2. A) Seventeen men received severe, traumatic brain injury secondary to motor vehicle (16) or skiing (1) accidents resulting in coma of 0 to 31 (mean, 5.8) days duration. Ten patients had associated medical problems consisting of fractures of the long bones (5), pulmonary contusions (3) and spinal cord injuries (2), but brain injury was their major medical problem. As expected they were significantly younger than the other two groups of men. B) Eleven men had documented myocardial infarctions, which were uncomplicated in all but one. No patient became hypotensive, none received sympathomimetics and two patients were treated with beta blockade. C) Seven men underwent elective surgery, 6 of whom had laminectomies. D) Seven young women (mean age 22.6 yr) were studied after traumatic brain injury (N = 5) or following intracranial vascular accidents (N=2). E) Twelve post menopausal women ranging in age between 50 and 84 years (mean, 67.8 years), who suffered intracranial vascular injuries, were evaluated to study the effects of brain injury on gonadotropin secretion in patients with nonfunctioning gonads. The median elapsed time (and ranges) between the acute event and the first post event hormonal observation for each patient group were: brain injury - men, 6.6 hr (0.7 to 50 hr); myocardial infarction, 3.6 hr (3.5 to 7 hr); elective surgery, 22.3 hr (4-26 hr); brain injury - young women, 3.8 hr (0.6 to 39 hr); brain injury postmenopausal women, 5.3 hr (1.3 to 19 hr).

Blood was obtained daily by our project nurse with additional samples retrieved from the Clinical Chemistry Laboratory. In order to test pituitary responsivity, gonadotropin releasing hormone (GnRH, 100 ug) was administered to 4 men with traumatic brain injury within 16 days of their accident and again following their recovery at least 3 weeks after their initial study. Serial blood samples were obtained for hormonal measurement by standard techniques. The percentage of testosterone not bound to circulating proteins was determined by ultrafiltration as follows. Plasma samples (0.6ml) were incubated at 37° C for 15 minutes with a dried aliquot of an ethanolic solution of H^3 testosterone (New England Nuclear Corp., Boston Ma), which had been previously purified on Lipidex-5000 (Packard Instruments, Downers Grove, Il). A 50 ul aliquot was removed for determination of total counts and the remainder transferred

TABLE 2

STUDY POPULATION

MEN

- HEAD TRAUMA N = 17
  - AGE RANGE 18 -64 yr.
    - MEAN 30.6 yr.
  - ASSOCIATED INJURIES
    - SPINAL CORD N = 2
    - PULMONARY CONTUSION N =3
    - BONE FRACTURES N = 5
    - NONE N = 7
- ELECTIVE SURGERY N = 7
  - AGE RANGE 35 - 75 yr.
    - MEAN 50.1 yr.
- MYOCARDIAL INFARCTION N = 11
  - AGE RANGE 37-66 yr.
    - MEAN 51.8 yr.

WOMEN

- PREMENOPAUSAL N = 7
  - HEAD TRAUMA N = 5
    - AGE RANGES 17-29 yr.
      - MEAN 22.6 yr
    - ASSOCIATED INJURIES
      - BONE FRACTURE N = 1
      - FACIAL INJURIES N = 1
  - INTRACRANIAL VASCULAR ACCIDENTS N = 2
    - AGE RANGE 30 - 45 yr
- POSTMENOPAUSAL
  - INTRACRANIAL VASCULAR ACCIDENTS N = 12
    - AGE RANGE 50 - 84 yr.
      - MEAN 67.8 yr.
    - ETIOLOGY
      - SUBARACHNOID HEMORRHAGE N = 6
      - INTRACRANIAL HEMORRHAGE N = 3
      - CEREBROVASCULAR ACCIDENT N = 1
      - HEAD TRAUMA N = 2

to a MPS micropartition chamber fitted with a YMB membrane (Amicon Corp., Danvers, Ma). After additional incubation at 37° C for 1 hour, the chambers were centrifuged for 7 minutes at 37° C and a 50 ul aliquot of the ultrafiltrate counted. A 50 ul aliquot of the patient's original plasma sample was counted for measurement of the background. The percentage of free testosterone was then calculated according to the following formula:

$$\text{free testosterone} = \frac{(\text{ultrafiltration counts} - \text{background counts})}{(\text{total counts} - \text{background counts})} \times 100$$

The mean $\pm$ 1 SD in 15 normal males was 2.34 $\pm$ 0.31 , while the intra- and interassay coefficients of variation were 3.1 and 5.2 respectively.

The effects of brain injury on the pituitary testicular axis became apparant within 24 hours of the accident (Figure 5). Testosterone levels were 413 $\pm$ 75 (SE) ng/dl twelve to 24 hours after injury compared to 535 $\pm$ 88 ng/dl on admission and continued to fall to 219 $\pm$ 46 ng/dl during the second day ($p<0.005$, compared to the initial value) and to 138 $\pm$ 22 ng/dl on the third day ($p<0.005$). The levels remained suppressed throughout the first eight days, irrespective of the level of consciousness as reflected in the Glasgow Coma Score (Figure 6). Two weeks to 8 months later, after the patient had recovered, their testosterone level returned to normal (629 $\pm$ 77 ng/dl). In the 9 patients treated with dexamethasone (16 - 24 mg/day), the testosterone nadir was comparable to that present in the nontreated patient (100 $\pm$ 12 vs 92 $\pm$ 35 ng/dl).

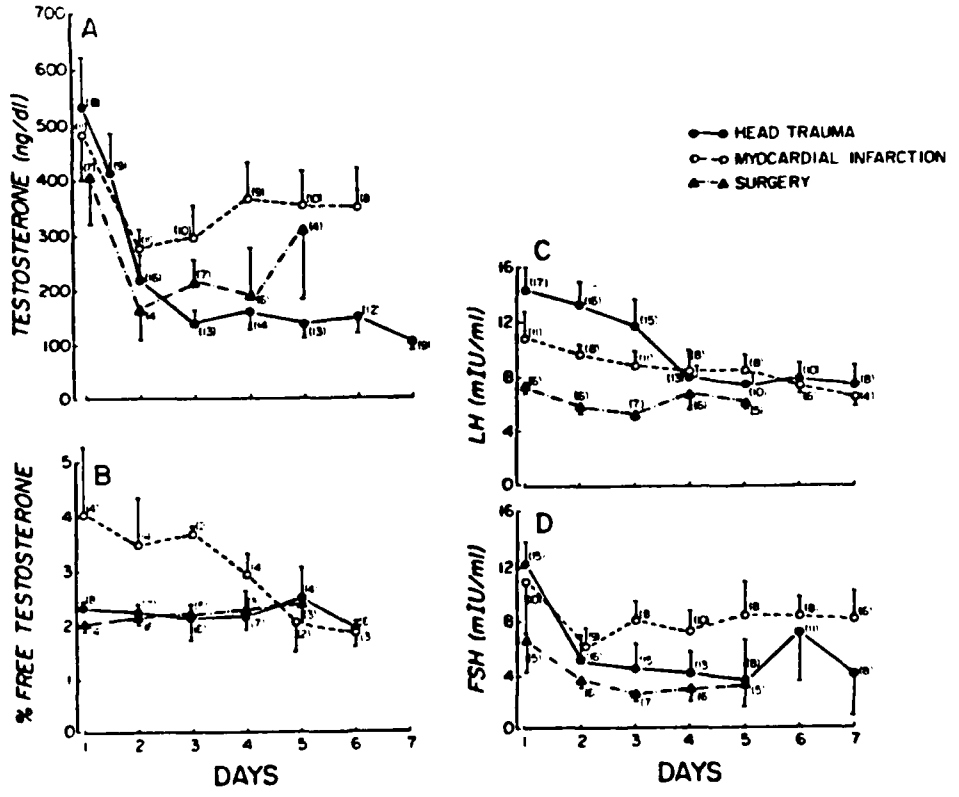

Figure 5. Mean testosterone (A), ultrafilterable testosterone (B), LH (C) and FSH (D) levels in patients having traumatic brain injury (o___o), acute myocardial infarction and (o--o) and elective surgery (Δ-·-Δ). The numbers in parentheses represents the number of samples assayed.

Despite the profound fall in total testosterone, the percent ultrafiltrable testosterone was constant ranging between 1.90 ± 0.065 and 2.52 ± 0.063 (Figure 5). Initially, sex hormone binding capacity was 0.54 ± 0.09 µg/dl and varied between 0.55 ± 0.05 and 0.49 ± 0.07 µg/dl.

Reductions in both gonadotropin concentrations were observed. LH levels were significantly lower by the fourth day (14.3 ± 2.0 vs 7.8 ± 1.0 mIU/ml, $p<0.025$), while

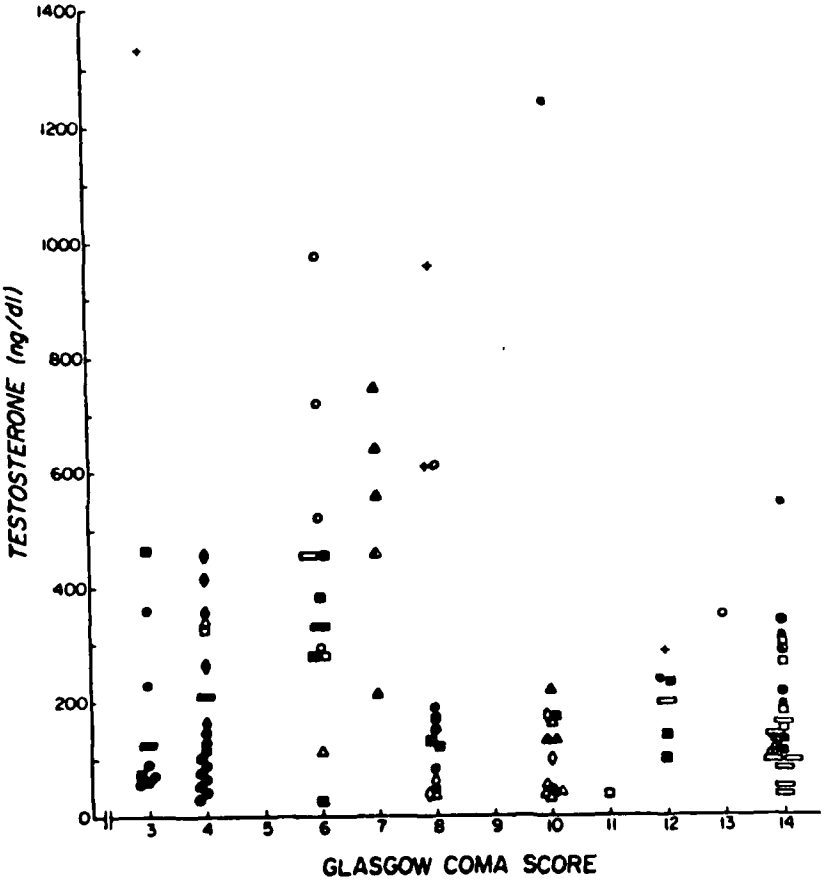

Figure 6. Correlation of circulating testosterone levels with neurological function as assessed by the Glasgow Coma Score in patients with traumatic brain injury revealed that testosterone concentrations remained low despite return to normal neurologic status. Each symbol represents a different patient.

FSH decreased from 12.0 ± 3.7 to 4.0 ± 1.8 mIU/ml ($p<0.05$). There were no significant differences in the magnitude of the fall in either gonadotropin between those patients who received dexamethasone and those who did not. The LH and FSH responses to GnRH administration during the acute phase were normal (Figure 7), despite the fall in basal hormone levels. In men undergoing elective surgery, testosterone levels were significantly lower on the first postoperative day (409 ± 92 vs 160 ± 51 ng/ml, $p<0.05$), without a corresponding alteration in either the percent ultrafiltrable testosterone (Figure 5) or SHBG. Although LH did not change, FSH was significantly ($p<0.02$) reduced within the first day. Testosterone and FSH levels remained below baseline for the duration of the observation period.

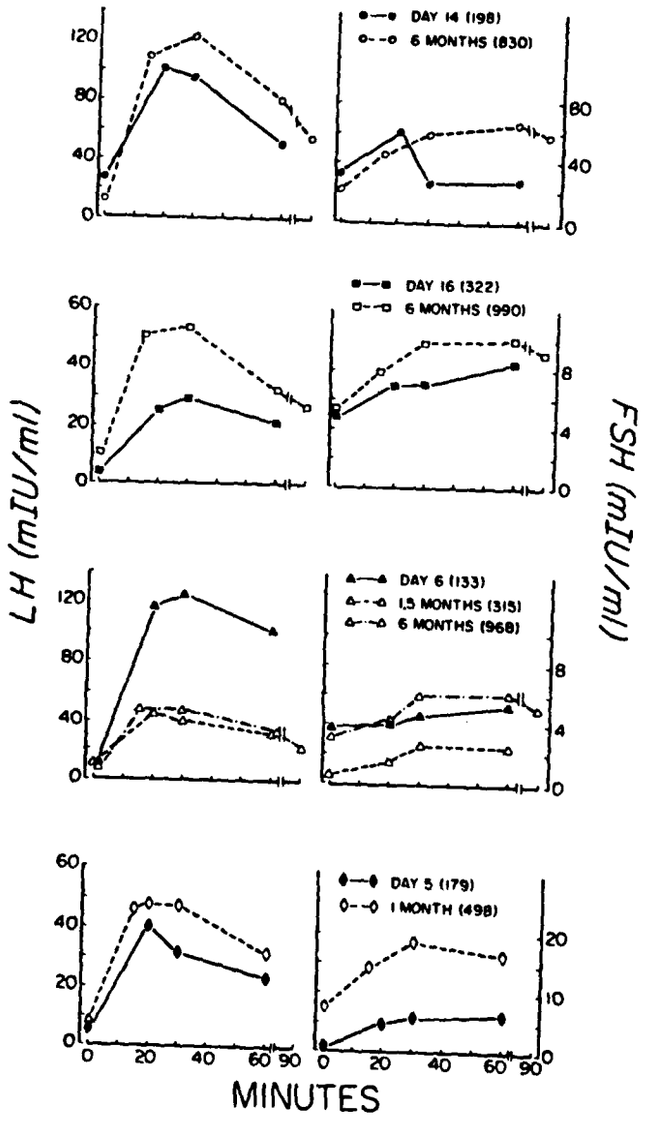

Figure 7. LH (left panel) and FSH (right panel) responses to GnRH (100 µg) during the acute and recovery phases of traumatic brain injury. Patients 1, 2, and 4 were receiving dexamethasone during their initial study. The number in the parenthesis represents the testosterone concentration on the initial sample of each study.

Myocardial infarction also altered the pituitary-testicular axis. Testosterone concentrations were significantly lower within one day of the onset of the illness (478 ± 76 vs 275 ± 35 ng/ml, $p<0.01$), but then stabilized between 350 and 370 ng/dl. Despite the 43% reduction in total testosterone there were no changes in any other parameter examined (Figure 5). The apparent fall in the percentages of ultrafilterable testosterone was due to very high initial levels in 2 of 5 patients.

WOMEN

Alterations in the pituitary-ovarian axis were also observed in young women (Figure 8). LH levels decreased by 66%, FSH by 64% and estradiol by 49% 24-48 hours after the acute event. Nadir levels were reached on days 4-6 and were 1.7 ± 0.3 compared

to 10.3 ± 4.6 mIU/ml, $p<0.05$ for LH, 0.3 ± 0.1 vs 3.8 ± 1.9 mIU/ml, $p<0.05$ for FSH and 40 ± 13 vs 200 ± 41 pg/ml, $p<0.005$ for estradiol. Sex hormone binding capacity was unaffected (baseline: 1.49 ± 0.18 µg/dl, estradiol nadir: 1.57 ± 0.11 µg/dl).

Decreases in mean LH and FSH levels in the postmenopausal women were observed within 24 hours of brain injury (Figure 9). Nevertheless, there were individual variations with some patients not demonstrating declines in gonadotropin levels until the third or fourth day. The nadir was reached by the sixth day at 27% and 43% of baseline for LH and FSH respectively. The apparent rise in estradiol by day 2 was secondary to the dramatic increase in 3 of 9 women (70 to 371, 85 to 114 and 23 to 394 pg/ml. Subsequently, the levels fell below baseline values to a mean nadir of 14.3 ± 4.8 pg/ml, $p<0.02$. Sex hormone binding capacity was 1.85 ± 0.13 µg/dl initially and did not change (1.68 ± 0.12 µg/dl on day of the nadir in estradiol levels).

Figure 8. Hormonal responses to brain injury in young women. The number in parenthesis represent the number of samples assayed.

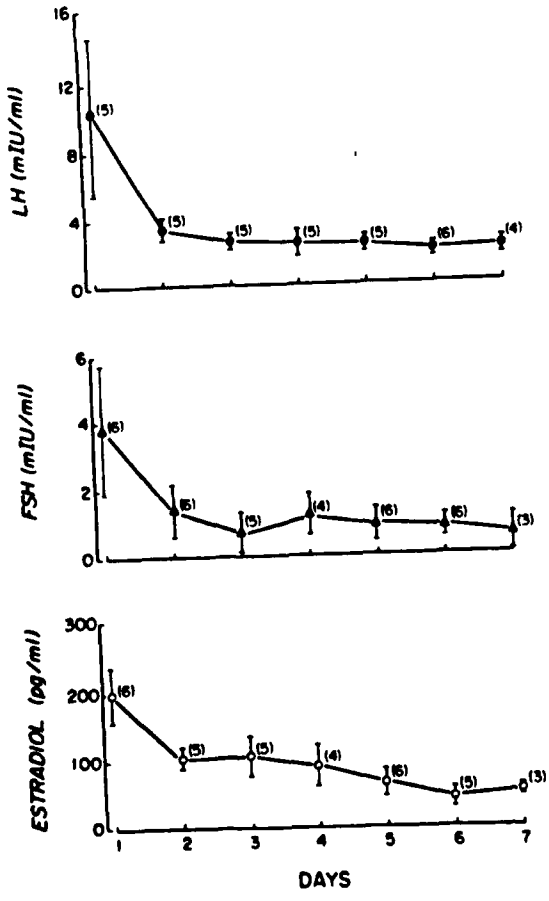

Fig. 8

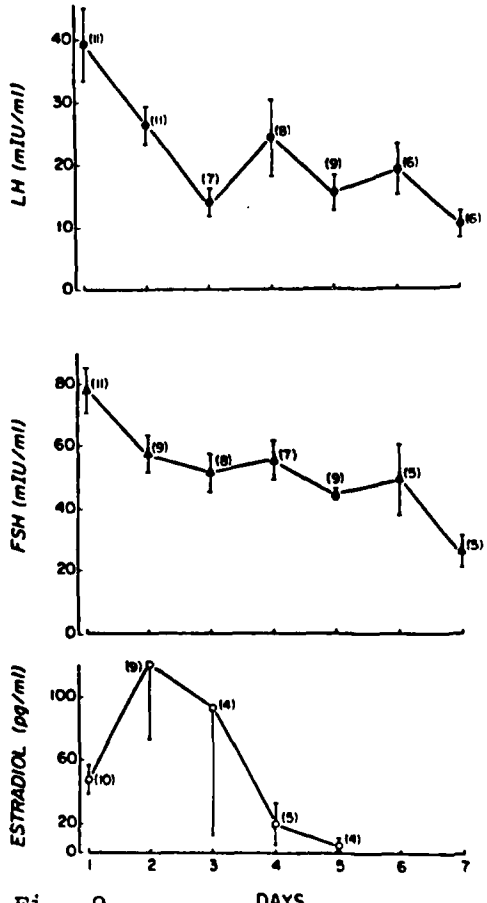

Fig. 9

Figure 9. Hormonal responses to brain injury in post-menopausal women. The numbers in parenthesis represents the number of samples assayed.

Our results indicated that gonadal function deteriorates in both men and women. In men testosterone concentrations fell by comparable amounts (head trauma, 271 ± 72; surgery, 195 ± 75; myocardial infarction, 202 ± 63 ng/dl), irrespective of the medical problem. Mean testosterone levels continued to decline in patients with brain injury and elective surgery. In the brain injured patients this represented a suppression to 8.3 to 62.4% (mean 28.9) of the baseline value. Testosterone remained suppressed well beyond the duration of the coma or the immediate postoperative period and returned to normal only after the patient had been ambulatory for several days to weeks. In contrast to our head trauma and surgical patients, the patients with uncomplicated myocardial infarctions reached their mean testosterone nadir on the second hospital day before starting to recover. Since these patients had a paucity of other complications, the smaller absolute decrease probably represented cessation of the stress rather than a qualitative difference in their response to the stress, especially since the initial decline was comparable to that observed in the other groups.

The ovary is also affected by systemic illness. Estradiol fell 75% in the young women and ultimately by 70% in the postmenopausal women. In the former, estrogen levels were significantly depressed by the first day and continued to fall into the postmenopausal range. Furthermore, these changes occurred irrespective of the timing of the menstrual cycle and were accompanied by falling gonadotropins. The early rise in mean estradiol levels in the postmenopausal women was due to increasing levels in only 3 of the women. Although this could have been due to preferential adrenocortical activation and conversion of cortisol precursors to estrogen, there were no significant differences in cortisol levels between the women whose estradiol rose (43.3 µg/dl) and those whose didn't (42.8 µg/dl). The changes in estradiol levels were not due to alterations in protein binding, since the sex hormone binding capacity did not change in either group.

Thus, **patients who are critically ill uniformly develop temporary hypogonadotropic gonadal insufficiency, irrespective of etiology.** In the male it is manifested by low testosterone levels which may fall into the female range, while estradiol levels in young women fall into the postmenopausal range. The low testosterone concentrations are not compensated by corresponding increases in the free fraction and are not due to reduced sex hormone binding capacity. Hypogonadotropism is also observed in the presence of a non functioning gonad. Although our studies do not completely establish the pathophysiology of this disorder, they do suggest a suprapituitary origin. The cause of the hypothalamic dysfunction is unclear and will be the subject of further investigation.

c. Development of a novel method for the measurement of free T_4.

Based upon the data presented in the preceding section demonstrating that acute brain injury causes temporary gonadal insufficiency, it was important to know whether other pituitary end organ functions were affected. There is a large body of data indicating that acute illness affects thyroidal function -- the so called "euthyroid sick" syndrome. However, there are conflicting data whether the free T_4 is high, normal or low, although the data by equilibrium dialysis (the gold standard) does not support a diagnosis of hypothyroidism. However, this method is tedeous and time consuming, and it is an expensive test to purchase from a commercial reference laboratory (approximately $35/sample). Although radioimmunoassays are available, they are not reliable for the differentiation of thyroid disease in a sick population.

Because of these limitations, a new method was developed using ultrafiltration methodology based upon the Amicon micopartition system. The assay is rapid and takes aproximately 4 hours for 25 determinations. The serum sample (50 µl) is diluted 1/10 in 0.1 M phosphate buffer, pH 7.4 and 0.3 ml of freshly purified ^{125}I-T_4 (45,000 cpm) is added. After an initial incubation at 37° for 15 min, an 0.6 ml aliquot is transferred to the MPS-I ultrafiltration device and equilibrated for an additional 30 min at 37°. Aliquots of the ultrafiltrate obtained after centrifugation and of the diluted serum sample are counted and the % free T_4 calculated by standard formulae.

The reliability and accuracy of this method was established as follows. The interassay coefficient of variation was determined using a serum sample assayed in 9 consecutive runs over a 4-month period. The mean of the sample was found to be 0.032 % with 1 SD of ± 0.003% , giving a coefficient of variation of 9.85 %. For determination of the intraassay coefficient of variation, 10 replicates were analyzed in the same assay. The mean ± SD % FT_4 was 0.016 ± 0.001 % , yielding a coefficient of variation of 7.24 %. During storage of the specimen at -20° for 4 months, the FT_4 concentration measured by ultrafiltration ranged between 0.026 % and 0.035% (0.32 ± 0.003 [± 1 SD]).

Comparison of our new procedure with established methods proved its utility. Twenty-five patient samples from normal, hyperthyroid, hypothyroid and pregnant subjects were assayed by equilibrium dialysis and compared to values obtained by our method (Figure 10). Regression analysis gave a slope of 0.89, a y -intercept of 0.16 ng/dl, and a correlation coefficient of 0.95. Comparison of the FT_4 results by

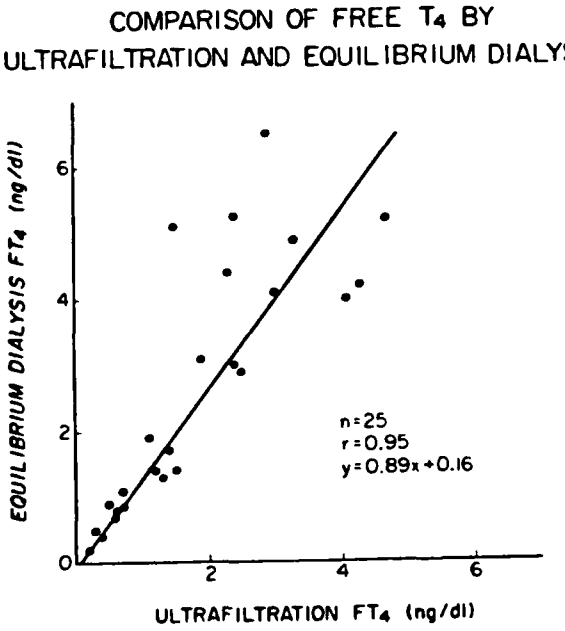

figure 10

ultrafiltration and RIA from 51 samples is shown in Figure 11. Regression analysis demonstrated a slope of 0.87, a y - intercept of 0.15 ng/dl, and a correlation coefficient of 0.88.

COMPARISON OF FREE T_4 BY
ULTRAFILTRATION AND RADIOIMMUNOASSAY

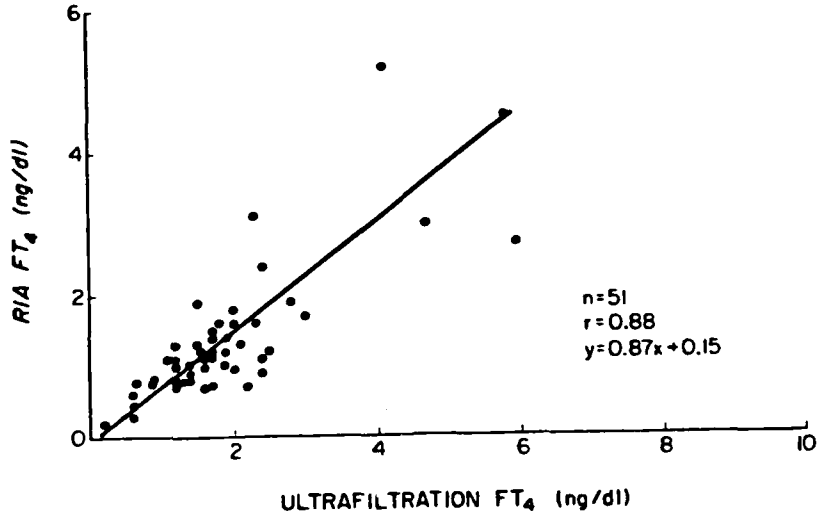

figure 11

In evaluation of the method, sera from 20 normal subjects, 12 hyperthyroid, 16 hypothyroid and 13 pregnant patients, as well as sera from 49 patients in the medical and surgical intensive care units were assayed. Mean $\pm$ 2 SD values (ng/dl) were: 1.66 $\pm$ 0.86 in normals, 3.91 $\pm$ 2.75* in hyperthyroid, 0.48 $\pm$ 0.58* in hypothyroid, 1.61 $\pm$ 0.64 in pregnant and 2.84 $\pm$ 0.132* in sick patients. Only one sick patient overlapped the hypothyroid range.

From these preliminary studies we feel that **we have demonstrated the clinical applicability of free T_4 determination by ultrafiltration.** The procedure is simple, rapid, economical and ideal for routine clinical assay, requiring only small volumes of sera. The procedure is reproducible, it correlates favorably with two established methods, equilibrium dialysis and radioimmunoassay, and it gives values which correlate with the thyroid status of the patient. Therefore, our preliminary studies suggests that FT_4 determination utilizing ultrafiltration with the MPS-I apparatus is able to substitute for the equilibrium dialysis method. Using our technique and a very sensitive TSH assay that is commercially available , we plan to study thyroid function in our head trauma population throughout the course of their illness as part of our investigation of the effects of acute brain injury on integrated neuroendocrine responses. The development of our method for free T_4 measurement have been submitted for publication.

d. Longitudinal study of pituitary function

Six patients receiving traumatic brain injury have been enrolled in the long term evaluation of the effects of traumatic brain injury on pituitary function. Under this protocol patients are admitted to the Clinical Research Center at 6 month intervals

*$p < 0.05$

for two sets of studies. As an initial screen, each subject receives the combination of TRH, GnRH and ACTH which tests the ability of the pituitary to respond to releasing hormones and the cortisol secretory capabilities of the adrenal which in turn is dependent upon adequate endogenous ACTH. Arginine is administered to stimulate growth hormone release. Basal testosterone, T_4 and T_3 levels are also measured. With the exception of adrenal secretion, all subjects have demonstrated completely normal pituitary function. Two subjects had borderline low morning cortisol concentrations during 20 minute sampling for three hours, but had normal adrenal stimulation to ACTH, suggesting partial ACTH insufficiency. These two subjects were readmitted to the CRC for administration of vasopressin, which releases ACTH directly and cortisol indirectly and an overnight metrapone study. Because of poor responses to one or both tests, they were readmitted to the CRC for a formal metyrapone study which was normal. Nevertheless, because of these abnormalities, both patients will be followed closely for signs of overt adrenal insufficiency. It is also apparant that we will need to perform similar studies in a comparable, but healthy cohort to more clearly define our normal ranges for our study of head trauma patients. Additional subjects as well as controls who received traumatic head injury, but without significant coma are presently being enrolled.

Papers, abstracts and presentations resulting from support by this contract.

1. Papers

Feibel, J., Kelly, M., Lee, L., and Woolf, P. Loss of adrenocortical suppression after acute brain injury: Role of increased intracranial pressure and brain stem function. J. Clin. Endocrinol. Metab. 57:1245-1250, 1983.

Shannon, N., and Woolf, P. A new method for the determination of free T_4 in serum using ultrafiltration techniques: Validation of the method and preliminary results. Submitted for publication.

Woolf, P.D., Hamill, R.W., McDonald, J.V., Lee, L.A., and Kelly, M. Apparent hypogonadism caused by critical illness: The "Hypogonadal Sick" syndrome. Submitted for publication.

2. Abstracts

*Woolf, P.D., Hamill, R.W., McDonald, J.V., Lee, L.A., and Kelly, M. Apparent hypogonadism caused by critical illness: The "Hypogonadal Sick" syndrome. Fifth Annual Traumatic Head Trauma Conference, Braintree, MA, October 17-19, 1984.

*Hamill, R.W., McDonald, J.V., Kelly, M., Lee, L., and Woolf, P.D. Acute Brain Injury: Sympathetic and adrenocortical responses. Fifth Annual Traumatic Head Trauma Conference, Braintree, MA, October 17-19, 1984.

*Woolf, P.D., McDonald, J.V., Kelly, M., Lee, L., Hamill, R.W. Neurohumoral responses to brain injury. Society for Neuroscience,

**Woolf, P.D., and Shannon, N. A new method for the determination of free T_4 in serum using ultrafiltration techniques: Validation of the method and preliminary results. 60th Annual Meeting of the American Thyroid Association, New York, New York, September 19-22, 1984.

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* Accepted for presentation

** Under consideration for presentation